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Genome Note

Whole-genome sequencing of a new sequence type (ST5352) strain of community-acquired methicillin-resistant *Staphylococcus aureus* from a hospital in Pakistan

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ABSTRACT

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important drug-resistant pathogen causing a number of diseases, resulting in increased mortality. Therefore, whole-genome sequencing of an MRSA strain isolated from a patient admitted to a hospital in Lahore, Pakistan, was performed to better characterise the strain and to understand the genetic components of antimicrobial resistance and virulence.

Methods: MRSA isolate Lr12 was sequenced on an Illumina HiSeq 2500 platform. The genome was assembled with SPAdes and was annotated using PGAP v.4.3. The strain was characterised using *spa*Typer 1.0, SCC*mec*Finder v.1.2 and MLST 2.0 server. Plasmids, antimicrobial resistance determinants and virulence factors were identified using PlasmidFinder v.2.0, CARD and VFDB, respectively.

Results: MRSA strain Lr12 has an estimated genome size of 2 769 144 bp with a GC content of 32.7% and harbours 1 plasmid, 2 prophages, 11 antimicrobial resistance determinants and several virulence factors. The allelic profile of seven housekeeping genes was unique and the sequence type (ST) was classified as unknown, hence a novel sequence type (ST5352) was assigned.

Conclusion: MRSA strain Lr12 has a novel sequence type (ST5352) and could be used as a reference strain for comparative genomic analysis of other MRSA strains belong to ST5352.

Staphylococcus aureus is an opportunistic pathogen causing numerous skin infections as well as systemic and life-threatening diseases [1]. *Staphylococcus aureus* is proficient at acquiring antimicrobial resistance genes and has developed resistance to many antibiotics. Methicillin-resistant *S. aureus* (MRSA) is virtually non-susceptible to all β-lactams and has been globally recognised as a major cause of hospital- and community-acquired infections [2]. MRSA is further characterised by sequence type (ST) using multilocus sequence typing (MLST), which indicates the genetic lineage and characteristics of strains from diverse geographical regions.

Here we report a draft genome of MRSA strain Lr12 having a novel MLST profile, isolated from a patient admitted at a hospital in Lahore, Pakistan. The isolate was cultured on mannitol salt agar (Oxoid Ltd.) at 37 °C and antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion method [1]. Genomic DNA (gDNA) was extracted using a DNA Extraction Kit (Invitrogen) and was quantified using a Qubit 2.0 fluorometer. gDNA libraries prepared using a Nextera XT Library Prep Kit (Illumina Inc., San Diego, CA, USA) were sequenced on an Illumina HiSeq 2500 platform (Illumina Inc.). Raw reads were trimmed using Trimmomatic 0.30 [3] and were de novo assembled using SPAdes v.3.7 [4]. De novo assembly generated 19 contigs, with the largest contig of 675 708 bp and an N_{50} value of 362 282 bp. The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v.4.3 was used for annotation, which determined a total genome length of 2 769 144 bp with 2859 coding sequences (CDSs), 2931 genes and 2755 coding genes. Strain Lr12 has a GC content of 32.7%, 59 tRNAs, 9 rRNAs (seven 5S and one each of 16S and 23S rRNAs) and 104 pseudogenes.

Molecular typing using *spa*Typer 1.0 (https://cge.cbs.dtu.dk/services/spatyper/) and SCC*mec*Finder v.1.2 (https://cge.cbs.dtu.dk/services/SCCmecFinder/) showed that strain Lr12 belongs to *spa*

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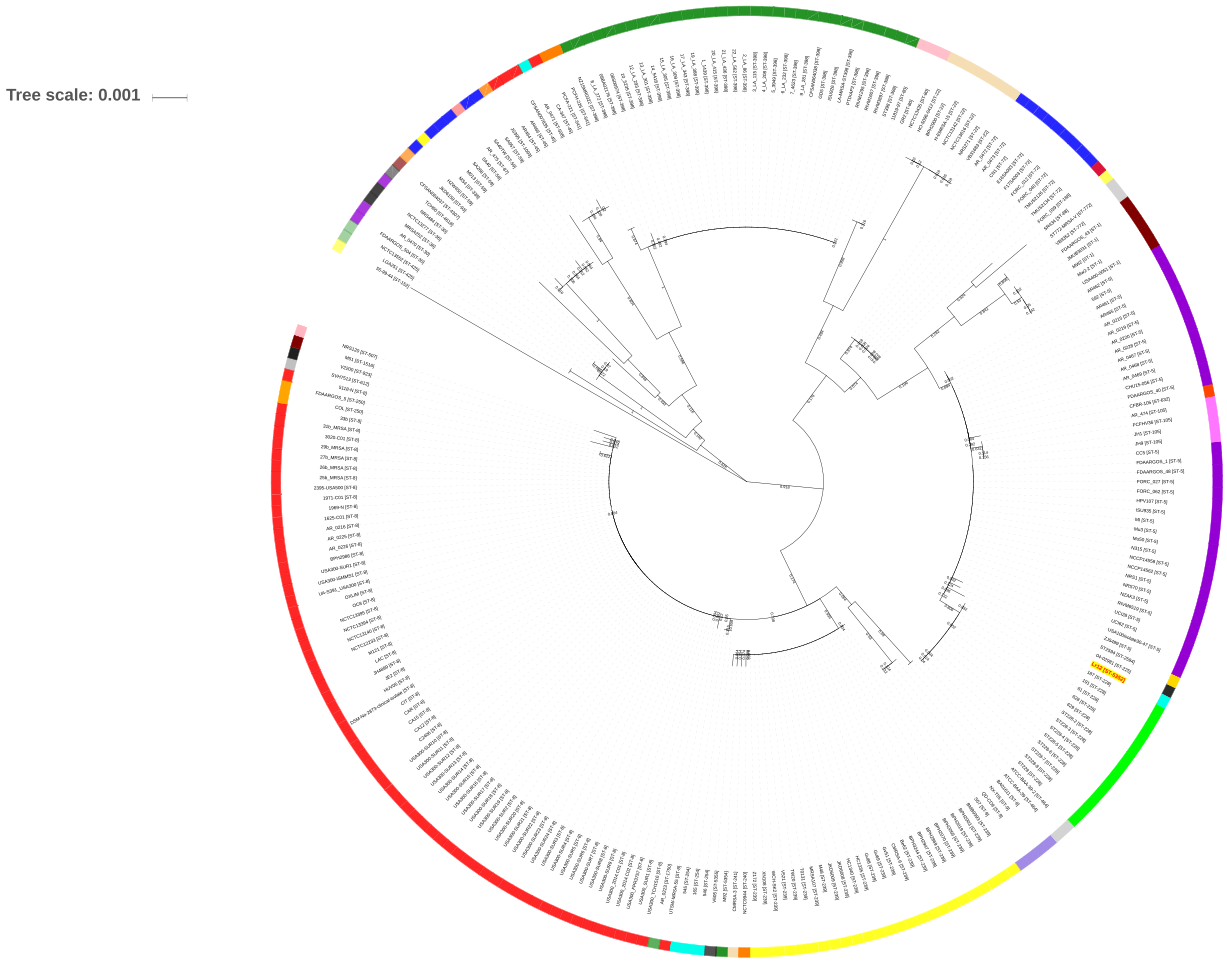

Fig. 1. A maximum likelihood tree based on variations in housekeeping genes of 251 global methicillin-resistant *Staphylococcus aureus* (MRSA) strains (among 397 *S. aureus* genomes available at NCBI at the time of analysis, 15 February 2019) and MRSA strain Lr12. Strain Lr12 is positioned in a separate branch and is highlighted in red, boldfaced and yellow background. The outermost coloured ring represents different sequence types (STs) and the tree was refined and annotated using iTOL (https://itol.embl.de/).

type t442, harbouring staphylococcal cassette chromosome *mec* (SCC*mec*) type V and is community-acquired MRSA (CA-MRSA). The MLST 2.0 server (https://cge.cbs.dtu.dk/services/MLST/) predicted a unique MLST profile and identified an unknown sequence type for CA-MRSA strain Lr12. Following verification, a novel sequence type (ST5352) was assigned by submitting the genome and its allelic profile of housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *yqi*) to the PubMLST database (https://pubmlst.org/saureus/). MLST phylogeny based on variations in seven housekeeping genes of all publicly available complete genomes of global MRSA at NCBI also confirmed the uniqueness of the MLST profile and positioned strain Lr12 in a separate branch (Fig. 1). PlasmidFinder v.2.0 (https://cge.cbs.dtu.dk/services/PlasmidFinder/) identified one plasmid replicon (*rep20*) of 23 118 bp in length, and PHASTER [5] detected two intact prophage regions, namely phiETA3_NC_008799 (49.5Kb) and JS01_NC_021773 (49.2Kb) with 100% identity.

The Comprehensive Antibiotic Resistance Database (CARD) [6] detected the methicillin resistance gene *mecA*, the aminoglycoside resistance gene *aac(6′)*-Ie–*aph(2")*-*Ia*, the glycylcycline resistance gene *mepA*, the trimethoprim resistance gene *dfrG*, the tetracycline resistance gene *tet(38)*, the *tet(38)* gene regulator *mgrA*, the two-component system regulators *arlS* and *arlR* conferring resistance to fluoroquinolones and the *mepA* resistance gene regulator *mepR* as well as point mutations in the genes *gyrA* and *parC* conferring resistance to fluoroquinolones. These results were highly correlated with antimicrobial susceptibility test results as the strain was resistant to ampicillin, methicillin, gentamicin, streptomycin, erythromycin, clindamycin, cefixime and meropenem but remained susceptible to tetracycline, linezolid, fusidic acid, chloramphenicol and vancomycin. The Virulence Factor Database (VFDB) [7] identified several virulence determinants, including autolysin (*atl*), α-haemolysin (*hly*/*hla*), fibrinogen- and fibronectin-binding protein (*efb* and *fnbA*), staphylococcal protein A (*spa*), exotoxins, leukocidin (*lukM*), Panton–Valentine leukocidin (*lukF*-PV and *lukS*-PV) and toxic shock syndrome toxin (*tsst*).

Accession numbers

The genome sequence of CA-MRSA ST5352 strain Lr12 has been deposited in NCBI GenBank with the accession no. **CP039162**. The raw data are available in the NCBI SRA (accession no. **SRR9694130**) under BioProject accession no. **PRJNA520768**.

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Competing interests

None declared.

Ethical approval

Not required.

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